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IMMUNE RESPONSE BY THE PRODUCTION OF ANTIBODIES AGAINST *Aeromonas hydrophila* BY *Cyprinus carpio* (Linn.)

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Fishes are always in a threat of being attacked by several pathogens including protozoa, fungi, algae, viruses, bacteria and parasites; as they are always present in the medium i.e. the water in which the fishes live. Bacterial diseases are responsible for heavy mortalities in both wild and farmed fishes. Several workers have shown that the normal bacterial flora of fish is a direct reflection of the bacterial population of the water in which they swim (Horsley, 1973; Sakata *et al*., 1980). These microorganisms get a chance to invade the tissues of fish, which are made susceptible to infection by stress factors or other disease process. The most significant microorganism in this respect is *Aeromonas hydrophila* (Allen *et al*., 1980).

Aeromonas hydrophila is widely distributed in the aquatic environment. The bacteria are found in clean as well as organically polluted fresh water and in marine system too except at the extreme salinity. The organism may be found in association with other pathogens, such as *A. salmonicida*, although there is no evidence for the presence of synergistic interactions leading to exacerbated disease conditions.

Most farmed and wild freshwater fishes are susceptible to infection by *Aeromonas hydrophila* but particularly cold water fish, such as brown trout *(Salmo trutta*), rainbow trout (*Onchorynchus mykiss*), Carp (*Cyprinus carpio*), catfish (*Clarius batrachus*) and gold fish (*Carassius auratus*) etc.

As the infectivity of bacteria depends on endotoxins released by dead bacteria or on exotoxins produced by living bacteria, the protection against infection will be determined by the development of antibodies or cell mediated immunity capable of neutralizing these toxins. Avtation, R.R in 1969 reported the effect of temperature on antibody production and immunological memory in carp, (*Cyprinus carpio*) immunized against bovine serum albumin (BSA).

In the present study fifteen fishes, *Cyprinus carpio* (Linnaeus) were collected from a collection site of Dal Lake. The fishes having an average weight of 250 grams were selected. Group one define with normal control, having five fishes. Group two divided into two subgroups having ten fishes. Each sub-group having five fishes (a) Healthy fishes subjected for primary response (b) Healthy fishes subjected for Secondary response. Fishes were reared in a well-managed rearing pond, provided with all the necessary conditions.

The swabbing was done from the infected fish suspected for bacterial infection and subjected for Gram's staining for bacterial classification. Our desired bacterial species was *Aeromonas hydrophila* and was culture on Rimler-Shotts media.

Antigen was prepared out of the bacterial culture by heat-killed method in PBS and 5% formalin and the healthy fishes were given the

doses of the antigen at the rate of 0.2 ml. After a weeks time the blood sample of the fishes was taken from group (a) and the serum collected after centrifugation was determined for primary response by Ouchterlony gel immunodiffusion. The second group was again injected with the same dose and checked for secondary response.

The study of primary and secondary immune responses gave a deep insight in understanding the impact of *A. hydrophila* bacteria on the antibody production in *Cyprinus carpio* Linnaeus. The results of the present study are in accordance with Mikryakov (2003) who studied the immune response to *A. hydrophila* and reported that acquired immunity in carp is some what similar to that of warm blooded animals and is based on the process of recognition, destruction, antigen metabolism, elimination of bacterial decomposition products and increase of antibody producing cell concentration. The carp immune system responded to intraperitoneal introduction of *A. hydrophila* with modification variability of immune cell and humoral factors accompanied by an increase of antigen reacting, antigen destroying, antibody synthesizing cell concentrations as well as their functional activity.

The rate of production of antibodies analysed, showed that there was a gradual increase in rate of antibody production from 7th day and abrupt rise was noticed from 5th week of post immunization. Ouchterlony gel immunodiffusion technique was used in the present study. More distinct precipitation bands were observed in the secondary immune 0.response than the primary response depicting that the antibody production was higher in the secondary response than the primary response. On the decrease of the temperature, the fishes responded with less production of antibodies and less distinct bands were observed. Therefore it is evident from the present study that the freshwater fish *Cyprinus carpio* responded well to the antigen stimuli provided to them with *Freund's complete adjuvant*.

REFERENCES

1. Allan, B.J. and Stevenson, R.M.W Extracellular virulence factors of *Aeromonas hydrophilla* in fish infections; *Canadian Journal of Microbiology*. **27**, 1114-1122 (1981)
2. Avtation, R.R. Temperature effect on antibody production and immunological memory in carp (*Cyprinus carpio*) immunized against bovine serum albumin (BSA). *J. Immun*. **17**: 927-931 (1969)
3. Horsley, (1973); Sakata *et al*., Normal bacterial flora of fish is a direct reflection of the bacterial population of the water in which they swim. *Canadian Journal of Microbiology*. **29**, 1125-1127 (1980)
4. Mikryakov, Soltani, M., V. R., Lapirova, T. B., Zabotkina, E. A. and Popov, A. V., Assessment of some immune response variables of immunized common carp (*Cyprinus carpio*) following exposure to organophosphate, malathion. *Bulletin of the European Association of Fish Pathologists*, **23**(1): 18-24 (2003)